OncoImmunology 2:11, e26329; November 2013; © 2013 Landes Bioscience

hTERT deficiency in naïve T cells affects lymphocyte homeostasis in myelodysplastic syndrome patients

Lili Yang[1,2], Erika A Eksioglu[3], and Sheng Wei[3,*]

[1]Department of Immunology; Tianjin Cancer Institute and Hospital; Tianjin Medical University; Tianjin, China;
[2]Key Laboratory of Cancer Immunology and Biotherapy; Tianjin, China; [3]Immunology Program at the H. Lee Moffitt Cancer Center; Tampa, FL USA

Keywords: bone marrow failure, myelodysplastic syndrome, telomere, T lymphocyte

Myelodysplastic syndromes (MDSs) are hematopoietic stem cell disorders with a high potential to develop into acute myeloid leukemia (AML). We have recently demonstrated that naïve T cells, but not memory T cells, from MDS patients exhibit a pronounced deficiency in the mRNA coding for the catalytic subunit of telomerase (hTERT). We discuss the importance of this finding for lymphocytic homeostasis in MDS patients.

Myelodysplastic syndromes (MDSs) are hematopoietic stem cell disorders characterized by ineffective hematopoiesis and associated with a 30% probability of progression into acute myeloid leukemia (AML).[1] The cells of MDS patients exhibited a pronounced erosion in telomeres, repetitive hexanucleotide (TTAGGG) regions that preserve chromosome integrity during cellular division, protect cells against premature senescence, and inhibit cell death. Such a shortening is among the earliest signs of the disease.[2,3] The enzyme responsible for maintaining telomeres, telomerase, plays a complex role in the regulation of tissue homeostasis as well as during oncogenesis, especially in the case of rapidly dividing cells such as hematopoietic cells. A few research groups have studied telomerase and its catalytic component, telomerase reverse transcriptase (TERT, best known as hTERT), in the context of MDS. Thus, while telomere attrition in myeloid cells is a confirmed and common finding among MDS patients, the status of telomeres in the T-cell compartment of these individuals was unknown, as was the possible link between this phenomenon to the pathogenesis of the disease.[4,5] T cells, which play an important role both as effectors and as regulators of adaptive immune responses, normally undergo a finite number of cell divisions until they reach a terminally differentiated state and enter replicative senescence.[6] The repair of telomeres is therefore crucial for T cells, owing to their high rate of turnover. Thus, T cells, notably naïve T cells after thymic involution, are particularly sensitive to changes in telomerase activity.[7,8]

Based on these premises, we focused on assessing the status of telomeres in T cells from MDS patients as well as on gaining a basic mechanistic understanding of the possible deregulation of hTERT in this setting (**Fig. 1**).[9] We first investigated the length of telomeres in T cells obtained from MDS patients and found significant telomere attrition as compared with the same cells from healthy subjects. Importantly, because the disease has an average age of onset of ~70 y, we stratified our results based on age and observed that T cells from MDS patients exhibit telomere shortening irrespective of patient age. Indeed, no significant differences were observed in terms of telomere length between T cells obtained from relatively young (< 65 y) MDS patients and T cells isolated in older (> 65 y) individuals affected by the disease. Both these patients subgroups harbored T cells with telomeres that were shorter than those of control T cells. These observations suggest that telomere attrition is an important component of the alterations that characterize the hematopoietic compartment of MDS patients, presumably developing before the onset of symptoms.

By means of 5-bromo-2-deoxyuridine (BrdU, to assess cell cycle progression), carboxyfluorescein *N*-succinimidyl ester (CFSE, to assess proliferation) and Telomerase RNA component (TREC, to assess the enzymatic activity of telomerase) assays, we observed that T cells from MDS patients exhibit a proliferative defect as compared with normal T cells. Such a deficiency was accentuated upon stimulation with anti-CD3/anti-CD28 antibodies and correlated with enzymatic defects in hTERT, but not with the length of telomeres. Perhaps, this may be explained with the fact that the telomeres of T cells from MDS patients are shorter than those of control T lymphocytes to being with.

Our next step was to assess whether telomere dysfunction originates from antigen

*Correspondence to: Sheng Wei; Email: sheng.wei@moffitt.org
Submitted: 08/29/2013; Accepted: 08/31/2013
Citation: Yang L, Eksioglu E, Wei S. hTERT deficiency in naïve T cells affects lymphocyte homeostasis in myelodysplastic syndrome patients. OncoImmunology 2013; 2:e26329; http://dx.doi.org/10.4161/onci.26329

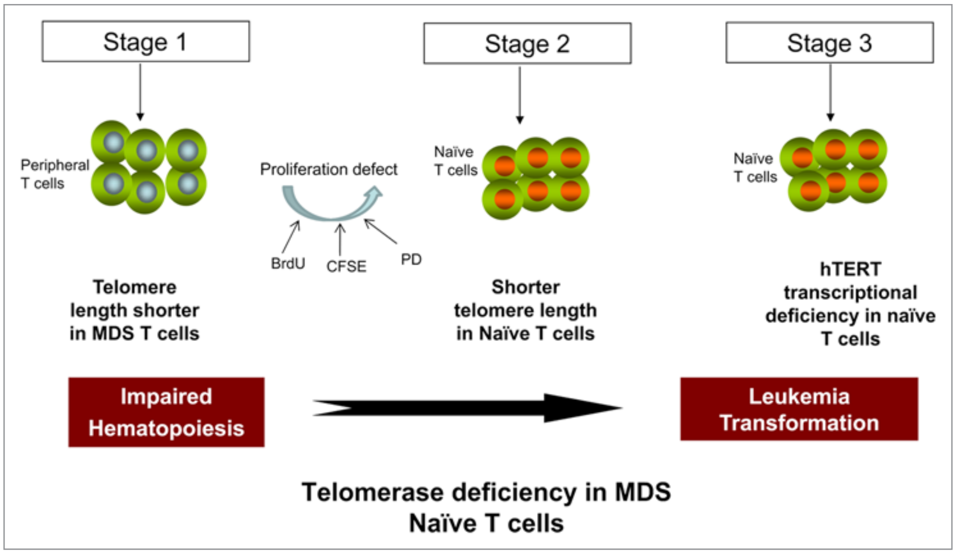

Figure 1. Telomerase deficiency in naïve T cells impacts lymphocytic homeostasis in myelodysplastic syndrome patients.

exposure. To this aim, were compared the length of telomeres in naive and memory T-cell populations from MDS cases and healthy individuals. The telomere length of naïve T cells was significantly decreased in MDS cases as compared with control subjects, whereas no difference was observed in memory T cells. This indicates that telomeric defects can affect specific T-cell subpopulations. Importantly, the telomerase activity of naïve T cells was significantly decreased in MDS patients as compared with healthy subjects, whereas memory T cells did not differ in this respect. These observations confirm that naïve T cells from MDS patients are specifically affected by telomeric defects and suggest that such defects are not related to antigen exposure. Moreover, these data partly explain our previous findings of a skewed naïve-to-memory $CD4^+$ T-cell ratio in MDS patients.[7]

We also detected that the inducible expression of the *TERT* mRNA is significantly decreased in the T-cell compartment of MDS patients as compared with that of healthy individuals, suggesting that the loss of telomerase activity that we observed is related to hTERT expression levels. However, we could not link such an MDS-associated attrition of naïve T-cell telomeres with mutations in the *TERT* promoter or with *TERT* copy number variations/deletions, suggesting that these naïve T cells do not derive from a malignant clone.[9] Other inflammatory diseases such as rheumatoid arthritis have been associated with defects in telomere repair, including reduced telomerase activity in naïve $CD4^+$ T cells due to insufficient induction of hTERT. Based on these premises, we compared the telomere length and telomerase activity of cells from MDS patients with those of cells from aplastic anemia (AA) patients.[10] We found a comparably lower telomerase activity in cells isolated from AA patients, defining MDS as a member of telomere repair disorders.

In summary, our study was the first to investigate the mechanisms for premature telomere attrition in the T-cell compartment of MDS patients, a phenomenon presumably brought about by a deficiency in telomerase selectively affecting (and hence hindering the regeneration of) naïve T cells. These findings explain previous data demonstrating skewed naïve-to-memory $CD4^+$ T-cell ratios in MDS patients, reflecting an altered lymphocyte homeostasis from a decreased naïve T-cell compartment and the accumulation of senescent cells. Future studies on the transcriptional regulation of hTERT, encompassing DNA methylation and histone modification assessments, will be important in order to elucidate the pathways underlying the attrition of telomeres in naïve T cells from MDS patients. Further insights into this issue will lead to a better understanding of the role of telomere abnormalities in the etiology of MDS, perhaps providing a basis for the development of novel therapeutic approaches against this disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed

References

1. Epling-Burnette PK, List AF. Advancements in the molecular pathogenesis of myelodysplastic syndrome. Curr Opin Hematol 2009; 16:70-6; http://dx.doi.org/10.1097/MOH.0b013e3283257ac7; PMID:19468267
2. Rollison DE, Epling-Burnette PK, Park JY, Lee JH, Park H, Jonathan K, Cole AL, Painter JS, Guerrier M, Meléndez-Santiago J, et al. Telomere length in myelodysplastic syndromes. Leuk Lymphoma 2011; 52:1528-36; http://dx.doi.org/10.3109/10428194.2011.568648; PMID:21635204
3. Wlodarski MW, Gondek LP, Nearman ZP, Plasilova M, Kalaycio M, Hsi ED, Maciejewski JP. Molecular strategies for detection and quantitation of clonal cytotoxic T-cell responses in aplastic anemia and myelodysplastic syndrome. Blood 2006; 108:2632-41; http://dx.doi.org/10.1182/blood-2005-09-3902; PMID:16614248
4. Effros RB, Pawelec G. Replicative senescence of T cells: does the Hayflick Limit lead to immune exhaustion? Immunol Today 1997; 18:450-4; http://dx.doi.org/10.1016/S0167-5699(97)01079-7; PMID:9293162
5. Gürkan E, Tanriverdi K, Başlamişli F. Telomerase activity in myelodysplastic syndromes. Leuk Res 2005; 29:1131-9; http://dx.doi.org/10.1016/j.leukres.2005.03.006; PMID:16111531
6. Effros RB. Telomerase induction in T cells: a cure for aging and disease? Exp Gerontol 2007; 42:416-20; http://dx.doi.org/10.1016/j.exger.2006.11.005; PMID:17182206

7. Zou JX, Rollison DE, Boulware D, Chen DT, Sloand EM, Pfannes LV, Goronzy JJ, Bai F, Painter JS, Wei S, et al. Altered naive and memory CD4+ T-cell homeostasis and immunosenescence characterize younger patients with myelodysplastic syndrome. Leukemia 2009; 23:1288-96; http://dx.doi.org/10.1038/leu.2009.14; PMID:19282834
8. Goronzy JJ, Fujii H, Weyand CM. Telomeres, immune aging and autoimmunity. Exp Gerontol 2006; 41:246-51; http://dx.doi.org/10.1016/j.exger.2005.12.002; PMID:16427234
9. Yang L, Mailloux A, Rollison DE, Painter JS, Maciejewski J, Paquette RL, Loughran TP, McGraw K, Makishima H, Radhakrishnan R, et al. Naive T-cells in myelodysplastic syndrome display intrinsic human telomerase reverse transcriptase (hTERT) deficiency. Leukemia 2013; 27:897-906; http://dx.doi.org/10.1038/leu.2012.300; PMID:23072779
10. Fujii H, Shao L, Colmegna I, Goronzy JJ, Weyand CM. Telomerase insufficiency in rheumatoid arthritis. Proc Natl Acad Sci U S A 2009; 106:4360-5; http://dx.doi.org/10.1073/pnas.0811332106; PMID:19255426